Amendments to the Specification:

Please replace paragraph beginning at line 18 on page 4 with the following amended paragraph:

-- It is therefore the primary object of the present invention to provide an isolated nucleic acid (*hCOLA1*) and the degenerate sequences thereof, which encodes human α1 chain collagen protein, comprising the nucleotide sequence set forth in ~~SEQ ID NO. 5~~ SEQ ID NO: 5. The present invention also provides the expression profile of the isolated collagen gene and the exact tissue and cellular localization of this collagen protein. Moreover, the present invention provides nucleotide fragments derived from ~~SEQ ID NO. 5~~ SEQ ID NO: 5 as a nucleic acid probe or primer. --

Please replace paragraph beginning at line 28 on page 4 with the following amended paragraph:

-- In one preferred embodiment, the present invention provides a novel human α1 chain collagen protein encoded by the nucleic acid mentioned above, which has the amino acid sequence set forth in ~~SEQ ID NO. 1~~ SEQ ID NO: 1. --

Please replace paragraph beginning at line 14 on page 5 with the following amended paragraph:

-- Yet still another aspect of the present invention provides a diagnostic kit for detecting the disease related to the mutation of ~~SEQ ID NO. 5~~ SEQ ID NO: 5 in a

mammal or human, comprising the nucleic acid probe or primer described above. --

Please replace paragraph beginning at line 4 on page 6 with the following amended paragraph:

-- FIG. 3 is a diagram showing the complete nucleotide sequence (~~SEQ ID NO. 5~~ SEQ ID NO: 5) and the corresponding amino acid sequence (~~SEQ ID NO. 1~~ SEQ ID NO: 1) of the human α1 chain collagen of the invention. --

Please delete paragraph beginning at line 14 on page 6.

Please replace paragraph beginning at line 21 on page 6 with the following amended paragraph:

-- ~~FIG. 7(A)~~ FIG. 6(A) is a Northern blot containing 2 μg of poly(A)$^+$ RNA from indicated tissues hybridized with human α1 chain collagen cDNA-specific probe; and human ß-actin-specific probe as an internal control. ~~FIG. 7(B)~~ FIG. 6(B) is a Northern blot containing 2 μg of poly(A)$^+$ RNA from indicated cardiovascular tissues hybridized with human α1 chain collagen cDNA-specific probe; and human glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) probe as an internal control. --

Please replace paragraph beginning at line 29 on page 6 with the following amended paragraph:

-- ~~FIG. 8~~ FIG. 7 is a quantitative RT-PCR of the expression of human α1 chain collagen from human fetal and adult tissues. Human glyceraldehyde 3-phosphate dehydrogenase was used as internal control. --

Please replace paragraph beginning at line 3 on page 7 with the following amended paragraph:

-- ~~FIG. 9~~ FIG. 8 is an ~~in situ~~ *in situ* hybridization analyses of expression of the human α1 chain collagen mRNA expression. Cardiovascular sections and cells were hybridized with digoxigenin labeled antisense riboprobes for human α1 chain collagen. (A) Longitudinal section, artery; (B) longitudinal section, ventricle; and (C) aortic smooth muscle cells. Control hybridizations labeled with sense probes did not produce signals (data not shown). *Bar*, 10 μm. --

Please replace paragraph beginning at line 11 on page 7 with the following amended paragraph:

-- ~~FIG. 10~~ FIG. 9 is a diagram showing the expression of the human α1 chain collagen protein in *E. coli*. ~~FIG. 10(A)~~ FIG. 9A shows SDS-PAGE analysis, wherein the numbers indicated are molecular weight standards; lane 1 is the non-induced cell lysate; lane 2 is the cell lysate induced by IPTG for 2 hours; and lane 3 is the cell lysate induced for 3 hours. ~~FIG. 10(B)~~ FIG. 9B, lane 1 shows the human α1 chain collagen protein purified by Ni-column and stained with Coomassie brilliant blue; and lanes 2 and 3 are

western blot detected by anti-histidine antibody, wherein lane 2 is the non-induced cell lysate and lane 3 is the cell lysate induced by IPTG for 2 hours without purification. --

Please replace paragraph beginning at line 23 on page 7 with the following amended paragraph:

-- ~~FIG. 11~~ FIG. 10 is a diagram showing RT-PCR of the recombinant expression of human α1 chain collagen in COS7 cells, wherein "-" refers to negative control; "+" is the RT-PCR products from transformants containing human α1 chain collagen gene; and numbers indicated are molecular weight standards. --

Please replace paragraph beginning at line 20 on page 8 with the following amended paragraph:

-- The nucleic acid sequence of the full-length human α1 chain collagen (*hCOLA1*) gene and the deduced amino acid sequence thereof are shown in FIG. 3 (~~SEQ ID NO. 5~~ SEQ ID NO: 5 and ~~SEQ ID NO. 1~~ SEQ ID NO: 1, respectively). The novel human α1 chain collagen gene comprises 2,865 bp, which encodes 954 amino acids with about 99,000 Da in molecular weight, and is located at the p11.2-12.3 on human chromosome ~~XI~~ VI. --

Please replace paragraph beginning at line 27 on page 8 with the following amended paragraph:

-- The above isolated nucleic acid (*hCOLA1* gene) comprises at least the nucleotide sequence set forth in ~~SEQ ID NO. 5~~ SEQ ID NO: 5 (including DNA and RNA sequences) or the complementary sequences thereto, and the genomic DNA sequence. Those skilled in this art will be aware that the nucleotide sequences can be modified in accordance with any method known in the art, and are also within the scope of the invention. For example, degenerate codons can be used to replace the relative positions but the gene encodes the same amino acid sequence. Further, additional codons can be inserted into the sequence or added either at the 3'- or 5'-end, but the activity of the protein is not affected or slightly affected. Accordingly, the complementary sequences and degenerate sequences of ~~SEQ ID NO. 5~~ SEQ ID NO: 5, and various modified variants are included in the present invention. See, for example, Sambrook, et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 1989. --

Please replace paragraph beginning at line 15 on page 9 with the following amended paragraph:

-- In accordance with the present invention, the human α1 chain collagen encoded by the isolated *hCOLA1* gene comprises three domains (as shown in FIG. 5), including (i) von Willebrand factor A domain (having amino acid sequence set forth in ~~SEQ ID NO. 2~~ SEQ ID NO: 2); (ii) thrombospondin N-terminal-like domain (having amino acid sequence set forth in ~~SEQ ID NO. 3~~ SEQ ID NO: 3); and (iii) collagenous domain (having amino acid sequence set forth in ~~SEQ ID NO. 4~~ SEQ ID NO: 4). To

analyze the primary sequence of the protein and to compare with other known 20 collagens, the human α1 chain collagen of the present invention belongs to FACIT family (fibril associated collagens with interrupted triple helices). From the domains described above, it is inferred that the physiological functions of the human α1 chain collagen of the invention may be involved in platelet aggregation, cell adhesion, and the activation of transformation growth factor. In addition, the N-terminal of the collagen protein further includes a signal peptide with 22 amino acids in length. It is inferred that the human α1 chain collagen of the invention is located in the extracellular matrix. --

Please replace paragraph beginning at line 14 on page 10 with the following amended paragraph:

-- In one preferred embodiment, the present invention provides a recombinant vector in which the *hCOLA1* gene is cloned into Bluescript KS (+) vector (Strategene). The recombinant vector is deposited at the Culture Collection and Research Center (Food Industry Research and Development Institute, 331 Shih-Pin Road, Hsinchu, Taiwan) on November 14, 2000, and assigned accession number CCRC 940331. --

Please replace paragraph beginning at line 20 on page 10 with the following amended paragraph:

-- One can produce the novel human α1 chain collagen protein which have the amino acid sequence set forth in ~~SEQ ID NO. 1~~ SEQ ID NO: 1 using the isolated nucleic acid described above by any suitable method in any suitable expression system known in

this art. Therefore, the method for producing the human α1 chain collagen protein is also within the scope of the present invention. --

Please replace paragraph beginning at line 27 on page 10 with the following amended paragraph:

-- One preferred expression system for the recombinant production of the collagen of the invention is in transgenic non-human animals, wherein the desired collagen may be recovered from the milk of the transgenic animal. Such a system is constructed by ~~operably~~ linking the DNA sequence encoding the collagen of the invention to a promoter and other required or optional regulatory sequences capable of effecting expression in mammary gland. Likewise, required or optional post-translational enzymes may be produced simultaneously in the target cells, employing suitable expression system operable in the targeted milk protein producing mammary gland cells. --

Please replace paragraph beginning at line 9 on page 11 with the following amended paragraph:

-- In one preferred embodiment of the present invention, the nucleic acid of ~~SEQ ID NO. 5~~ SEQ ID NO: 5 is subcloned into an expression vector to obtain another recombinant vector. A suitable host cell (for example, eukaryotic or prokaryotic cell) is then transformed or transfected with the recombinant vector. The transformed or transfected cells are then cultured under the conditions sufficient for expression of human α1 chain collagen protein. Finally, the expressed proteins are recovered and purified.

Those skilled in this art will appreciate that the recovering and purifying method is not limited, for example, by various chromatographies. Preferably, the human α1 chain collagen is expressed using histidine tag fusion protein technique, and the recovering and purifying method is performed by affinity column. --

Please replace paragraph beginning at line 19 on page 12 with the following amended paragraph:

-- In one aspect of the present invention, the isolated nucleic acid further includes the fragments derived from ~~SEQ ID NO. 5~~ SEQ ID NO: 5 or the complementary sequences thereto to be as a nucleic acid probe or primer for detection. Those skilled in the art will be aware that the length of the nucleic acid fragment is not limited. For example, as a nucleic acid probe, the fragment preferably comprises at least 500 contiguous nucleotides in length derived from ~~SEQ ID NO. 5~~ SEQ ID NO: 5 or more, while as a nucleic acid primer, the fragment preferably comprises at least 20 contiguous nucleotides in length derived from ~~SEQ ID NO. 5~~ SEQ ID NO: 5 or more. The selection of the length of fragment is dependent upon the conditions of detection method as described below. For example, the temperature and ionic strength used in hybridization, or the temperature used in polymerase chain reaction (PCR). Generally, the length of the nucleic acid probe is in proportion to the specificity of the detection result. Accordingly, the nucleic acid probe preferably comprises at least 500 contiguous nucleotides in length derived from ~~SEQ ID NO. 5~~ SEQ ID NO: 5, and more preferably comprises the full-length nucleic acid of ~~SEQ ID NO. 5~~ SEQ ID NO: 5. In addition, the length of the

nucleic acid primer is in proportion to the specificity of the detection result. Accordingly, the nucleic acid primer preferably comprises at least 20 contiguous nucleotides in length derived from ~~SEQ ID NO. 5~~ SEQ ID NO: 5, and more preferably comprises 20-25 contiguous nucleotides, thereby increasing the specificity of the detection result. --

Please replace paragraph beginning at line 14 on page 15 with the following amended paragraph:

-- A Clontech SMART RACE cDNA Amplification kit was used to clone *hCOLA1* cDNA. Sequence specific primers used for the following RACE reactions were either deduced from the previously published partial human genomic clone 682J15 (Genbank Accession No. AL034452) or the cloned *hCOLA1* cDNA fragment. Initially, first strand cDNA was synthesized from 1 μg of total RNA pool (Clontech) using Superscript II reverse transcriptase with a specific primer 5'-GGTTCACCTTTGCTTCCCTTAG-3' (SEQ ID NO: 6), deduced from the clone 682J15. The reaction was following to the manufacture's protocol. The above reverse transcription reaction mixture was used for 5'RACE reaction with a sequence specific primer (5'-TTGGCCCATTAATCCTCGGTTTC-3' (SEQ ID NO: 7)), corresponding to nucleotides 1823-1845 of the *hCOLA1* cDNA and the universal primer provided by the kit. All assays were performed in a 50-μl reaction volume using the GeneAmp PCR system 9600 (Perkin-Elmer Cetus). --

Please replace paragraph beginning at line 1 on page 16 with the following amended paragraph:

-- To obtain the entire coding region of *hCOLA1* gene, first strand cDNA was synthesized from 1 μg of total RNA pool (Clontech) using Superscript II reverse transcriptase with an oligo dT primer. After reverse transcription, 1 μl of the reaction mixture was used for PCR amplification with a upstream primer (5'-ATTCCTGGGCCACCTGGTCCGATA-3' (SEQ ID NO: 8)), corresponding to the most 5' candidate initiator methionine of the clone 708F5 (Genbank Accession No. AL031782) and a downstream primer (5'-CTAATAGTTTGGTCCTTTTCT–3' (SEQ ID NO: 9)), corresponding to the 3' stop codon of the clone 682J15. A single band with a molecular size of 2.9 kilo bases was obtained (Figure 1). The band was excised from gel and cloned into the BlueScript II KS(+) vector (Strategene). The recombinant vector was deposited at the Culture Collection and Research Center (Food Industry Research and Development Institute, 331 Shih-Pin Road, Hsinchu, Taiwan) and assigned accession number CCRC 940331. After nucleotide sequence analysis, the PCR product was found to contain the entire open reading frame of *hCOLA1*. --

Please replace paragraph beginning at line 21 on page 16 with the following amended paragraph:

-- Nucleotide sequencing was carried out with the Sanger dideoxynucleotide chain termination method (Sambrook, et al., 1989). The sequence samples were prepared using the Ampli Taq cycle sequencing kit (Perkin–Elmer, Inc.) following the

manufacturer's protocol. The samples were applied to a 377 automatic sequencer (Perkin–Elmer, Inc.). All reported sequences were confirmed by sequencing of both sense and antisense strands. The full-length nucleotide sequence (~~SEQ ID NO. 5~~ SEQ ID NO: 5) and the deduced amino acid sequence (~~SEQ ID NO. 1~~ SEQ ID NO: 1) of the human α1 chain collagen of the invention is shown in FIG. 3. --

Please replace paragraph beginning at line 5 on page 17 with the following amended paragraph:

-- The human multiple tissue and the cardiovascular Northern blots, containing 2 μg of poly(A)$^+$ RNA from indicated tissues, were obtained from Clontech (catalog number 7780-1 and 7791-1, respectively). The blot was hybridized with a randomly primed ^{32}P-labeled probe corresponding to nucleotides 1236-1863 of the *hCOLA1* open reading frame at 60°C in ExpressHyb solution for one hour and washed with 2× SSC/0.1% SDS two times for 15 min each at 60°C. Then the blot was washed with 0.2× SSC/0.1% SDS three times for 15 min each at 60°C. Human *ß actin* or *GAPDH* probe was used as a control for the amount of RNA in each lane. As shown in Fig. ~~7A~~ 6A, a transcript of approximately 4.3 kb is observed, in agreement with the size of the cloned cDNA. The expression of *hCOLA1* collagen is mostly confined to placenta and heart tissues, with lower levels in skeletal muscle, small intestine, liver and lung. Another transcript of approximately 2.4 kb was detected to be hybridized with the probe in heart tissue. It probably is a splicing variant of the *hCOLA1* gene. We further examined the expression pattern of *hCOLA1* in human cardiovascular tissues containing fetal heart and

adult heart tissues, together with the aortic and cardiac tissues by Northern blot analysis. Surprisingly, the *hCOLA1* transcripts were only present in fetal heart and aortic tissues (Fig. ~~7B~~ 6B). Moreover, the 2.4 kb short transcript was only present in the fetal heart. Another 7.3 kb band was detected in both tissues. We do not know if this is an additional splicing variant of the *hCOLA1* gene. No hybridization signal was detected in adult heart and cardiac tissues. Although the result showing the absence of *hCOLA1* transcript in adult heart is inconsistent with the data of Northern blot analysis in Fig. ~~7A~~ 6A, the *hCOLA1* mRNA level in fetal heart is 22-fold in excess of the adult heart based on the quantitative RT-PCR results in Fig. ~~8~~ 7 (see below). The presence of the *hCOLA1* transcripts in aorta suggests that this novel collagen is derived from blood vessels. --

Please replace paragraph beginning at line 13 on page 18 with the following amended paragraph:

-- Five micrograms of total RNAs from a variety of human fetal and adult tissues obtained from Clontech (catalog number K4005-1) were used for reverse transcription reactions with oligo (dT) primers. After reverse transcription reactions, the relative quantity of endogenous *GAPDH* mRNA in each tissue sample was determined with CYBR Green fluorescence dye (Molecular Probes) using Real-time PCR analysis (LightCycler, Roche Molecular Biochemicals). The resulting *GAPDH* mRNA value in each tissue sample was used to normalize the sample for differences in the amount of total RNA added to each PCR reaction. Each of the normalized tissue samples was then split to perform the target ~~α1(XXI)~~ hCOLA1 collagen and control *GAPDH* amplifications

by Real-time PCR analysis. The relative quantity of ~~α1(XXI)~~ hCOLA1 collagen cDNA in each reaction was determined in the exponential phase to ensure that the amount of product amplified reflects the quantity of starting mRNA. Primers used for PCR amplifications are as follows: *GAPDH* (5'-TGAAGGTCGGAGTCAACGGATTTGGT-3' (SEQ ID NO: 10) and 5'-CATGTGGGCCATGAGGTCCACCAC-3' (SEQ ID NO: 11); 983-bp fragment); *hCOLA1* collagen (5'-TTCCTGGAAACCGAGGATTAATG-3' (SEQ ID NO: 12) and 5'-AGTCCACGATCACCCTTGTCAC-3' (SEQ ID NO: 13); 1546-bp fragment). Meanwhile, samples at a PCR cycle in the linear range of amplification (30 cycles for *hCOLA1*; 20 cycles for *GAPDH*) were electrophoresed on 1.5% agarose and stained with ethidium bromide for visualization. As shown in Fig. ~~8~~ 7, when normalized to the *GAPDH* values, the relative amounts of *hCOLA1* transcripts were 2.7, 22 and 30 times more in fetal brain, heart and liver than in the adult counterparts, respectively. The results indicate that *hCOLA1* expression is developmentally regulated and suggest a role for ~~α1(XXI)~~ hCOLA1 collagen in developmental processes in multiple tissues. Comparison of the *hCOLA1* expression in different adult tissues reveals that high levels of *hCOLA1* expression were detected in trachea, testis, uterus, and placenta, with modest levels of expression in brain, lung, colon, prostate, spinal cord, and salivary gland. The *hCOLA1* collagen mRNA expression was very low or undetectable in adult heart, liver, kidney, bone marrow, spleen, thymus, skeletal muscle, and adrenal gland. --

Please replace paragraph beginning at line 4 on page 21 with the following amended paragraph:

-- The entire coding region of the *hCOLA1* cDNA was amplified by PCR with primers 5'-ATGGCTCACTATATTACATTTCTC-3' (SEQ ID NO: 14), corresponding to the 5' cDNA region and 5'-TTAGTGATGGTGATGGTGATGCTCATAGTTT GGTCCTTTTCTG–3' (SEQ ID NO:15), corresponding to the 3' region including 6 histidine residues right before the stop codon. The amplified DNA construct was gel purified and sub-cloned into the expression vector pET 15b (Novagen) in which the Nco I site was digested and blunted with Klenow fragment. The recombinant protein was obtained by expressing the constructs in *E. coli* strain BL21 (DE3). The transformed *E. coli* was cultured in LB medium containing 100 μg/ml of ampicillin at 37°C to reach an optical density of 0.7 at 600 nm, followed by induction with IPTG at a final concentration of 1 mM and kept culturing for an additional 2 or 3 hours. The cell lysate with total proteins was analyzed by SDS-PAGE. The result is shown in FIG. ~~10A~~ 9A. --

Please replace paragraph beginning at line 21 on page 21 with the following amended paragraph:

-- One liter of the IPTG induced *E. coli* cells was cultured for 2 hours and then centrifuged at 5000 xg for 30 min. The cell pellet was washed with PBS and centrifuged again. The cell pellet was then re-suspended in 20 ml of PBS containing 1 mM of PMSF. The cell suspension was subjected to ultrasonication to break the cell walls. The cell lysate was then centrifuged at 30,000 xg for 40 min. The supernatant was applied to a Ni-agarose column (5 ml in bed volume) that has been equilibrated with 50 mM of Tris-HCl buffer, pH 8.0 at a flow rate 0.5 ml/min. The column was washed with the same

buffer containing 40 mM of imidazole. The recombinant *hCOLA1* was eluted with the same buffer containing 0.25 M imidazole. The eluate was quantified and analyzed by SDS-PAGE, followed by staining with Coomassie brilliant blue. A protein band with 98 kDa in molecular weight was observed on the gel (FIG. ~~10(B)~~ 9(B), lane 1). In addition, the proteins without purification were blotted to a PVDF membrane. An antibody to histidine tag (Clontech) was used to detect the recombinant protein. The result of Western blot is shown in FIG. ~~10(B)~~ 9(B), lanes 2 and 3, in which the band indicated at 98 kDa corresponds to the human α1 chain collagen protein of the invention. --

Please replace paragraph beginning at line 15 on page 22 with the following amended paragraph:

-- The *hCOLA1* cDNA containing entire open reading frame prepared by Example 4 was gel purified and sub-cloned into the expression vector pcDNA 3.1 containing CMV promoter (Invitrogen) in which the Pme I site was digested and blunted with Klenow fragment. The mammalian cells COS7 were transfected with the expression vector via Superfect (Qiagen), and cultured in DMEM supplemented with 10% FBS (Life Technologies) for 48 hours. About 10^6 cells were used for the extraction of total RNA. The reverse transcription was performed with oligo dT primer using 0.2 μg RNA as template. After reaction, PCR was carried out with primers T7 and BGHrev on the pcDNA3.1 vector using 0.5 μl solution. The result is shown in FIG. ~~11~~ 10, indicating that the vector is expressed in the transfected mammalian cells. --

Please replace paragraph beginning at line 6 on page 23 with the following amended paragraph:

-- The amino acid sequence of the human α1 chain collagen protein of the invention is compared with those of other 20 known collagens, particularly type IX and type XIX, the most similar in structures. The amino acid sequence identity of collagens between type IX and *hCOLA1* of the invention is 24%, while that between type XIX and *hCOLA1* of the invention is 27%, indicating that *hCOLA1* of the invention is a novel form of collagen ~~(FIG. 6)~~.

Amendments to the Sequence Listing:

Please substitute the Sequence Listing on pages 24 to 28 of the specification with the enclosed replacement Sequence Listing. A Statement Under 37 C.F.R. §§1.821 and 1.825, a disk containing the replacement sequence listing, and a paper copy of the replacement sequence listing accompany this response. The Sequence Listing has been amended to add 11 primer sequences as indicated in Examples of the application.

Attachment: Disk containing the replacement sequence listing (Attachment 1);

Statement Under 37 C.F.R. §§1.821 and 1.825 (Attachment 2);

Paper copy of the sequence listing in the disk (Attachment 3);

Replacement sheets (pages 24-30) reflecting changes in sequence listing (Attachment 4).

Amendments to the Drawings:

The attached 5 sheets of drawings include renumbering and changes made to Figs. 7-10 as renumbered. Fig. 6A-6C have been deleted. Figs. 7A, 7B, 9A, 9B, 10A, and 10B have been amended to indicate the bands of hybridization or expression products as stated in the specification.

Attachments: Replacement Drawing Sheets (Attachment 5)

Annotated Sheets Showing Changes in Red (Attachment 6)